

Patent claims

1. A screening method for detecting amidase or nitrile hydratase activity, which comprises
 - a) preparing on a suitable support a replica of cell colonies which express amidases or nitrile hydratases and which have grown on a suitable, solidified medium, followed by,
 - b₁) in the case of colonies having nitrile hydratase genes to be assayed, firstly incubating the cells adhering to the support with a substrate solution composed of a nitrile of the formula (I) $R-CN$ in which R is a C_1-C_{20} -alkyl, C_6-C_{20} -aryl or C_5-C_{20} -heteroaryl radical which is unsubstituted or mono- or poly-substituted with substituents inert under the reaction conditions and of a buffer, and subsequently
 - b₂) carrying out an incubation with a buffered hydroxylammonium salt solution, or,
 - c) in the case of cell colonies having amidase genes to be assayed, incubating the colonies adhering to said support with a substrate solution composed of an amide of the formula (II) $R-CONH_2$, in which R is as defined above, a hydroxylammonium salt and a buffer, and then,
 - d) following b₂) or c), staining the active colonies by transferring the support to an iron (III) salt solution, and
 - e) isolating, where appropriate, cells of said active colonies from said support or from the original areas in which said colonies have been present on the solidified medium.
2. The screening method as claimed in claim 1, wherein the support used is membranes or filter paper.
3. The screening method as claimed in claim 1, wherein in the substrate solutions in step b₁) nitriles of the formula (I) $R-CN$ and in step c) amides of the formula (II) $R-CONH_2$ are used, where R is a saturated or mono-unsaturated, linear,

branched or cyclic C₁-C₁₂-alkyl radical or a phenyl, biphenyl or naphthyl radical or a pyrrolidiny radical, which may be unsubstituted or mono- or poly-substituted with substituents from the group consisting of halogen, hydroxy, cyano, amino, unsubstituted or substituted phenyl or naphthyl, C₁-C₆-alkyl, C₁-C₆-alkoxy, C₁-C₆-alkylthio or phenoxy.

4. The screening method as claimed in claim 1, wherein the hydroxylammonium salt used is a chloride or a sulfate.
5. The screening method as claimed in claim 1, wherein the buffer used is phosphate buffer.
6. The screening method as claimed in claim 1, wherein the incubation solutions in step b₂) and c) contain 0.5 –2 mol/l hydroxylammonium salt in from 50 to 200 mM buffer.
7. The screening method as claimed in claim 1, wherein, where appropriate, a cosolvent of the group DMSO, DMF or C₁-C₄-alcohol is added to the substrate in step b₁) and in step c).
8. The screening method as claimed in claim 1, wherein the iron(III) salt solutions used in step d) are strongly acidified ferric chloride, ferric sulfate or ferric nitrate solutions or solutions of complex iron(III) salts from the group consisting of ferric ammonium citrate and ferric ammonium sulfate.
9. The screening method as claimed in claim 1, wherein an amidase is added to the screening solution in step b₁) for detecting nitrile hydratase activity.
10. The screening method as claimed in claim 1, wherein an amidase chromosomally integrated into an *E. coli* strain is used for detecting nitrile hydratase activity

expressed from vectors.

11. The use of the screening method as claimed in claim 1 for detecting amidase activity when cloning an amidase gene from appropriate organisms.
12. The use of the screening method as claimed in claim 1 for detecting amidase activity when cloning an amidase gene from bacteria.
13. The use of the screening method as claimed in claim 12, wherein the bacteria used are *Rhodococcus*, *Pseudomonas* or *Acinetobacter* bacteria.
14. The use of the screening method as claimed in claim 1 for detecting nitrile hydratase activity when cloning a nitrile hydratase gene from appropriate organisms.
15. The use of the screening method as claimed in claim 1 for detecting nitrile hydratase activity when cloning a nitrile hydratase gene from bacteria.
16. The use of the screening method as claimed in claim 15, wherein the bacteria used are *Rhodococcus*, *Pseudomonas* or *Acinetobacter* bacteria.
17. The use of the screening method as claimed in claim 1 for detecting increased amidase activity in random mutagenesis libraries.
18. The use of the screening method as claimed in claim 1 for detecting increased nitrile hydratase activity and stability in random mutagenesis libraries.
19. The use of the screening method as claimed in claim 1 for isolating nitrile hydratase genes from gene banks in *E. coli* plasmids.